Genomic Analysis of the *Snn1* Locus on Wheat Chromosome Arm 1BS and the Identification of Candidate Genes

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Abstract

The pathogen *Stagonospora nodorum* produces multiple host-selective toxins (HSTs) that induce cell death and necrosis in sensitive wheat (*Triticum* sp.) genotypes. One such HST is SnTox1, which interacts with the host gene *Snn1* on wheat chromosome arm 1BS to cause necrosis leading to disease susceptibility. Toward the positional cloning of *Snn1*, we developed saturated and high-resolution maps of the *Snn1* locus and evaluated colinearity of the region with rice (*Oryza sativa* L.). An F_2 population of 120 individuals derived from 'Chinese Spring' (CS) and the CS–*T. dicoccoides* chromosome 1B disomic substitution line was used to map 54 markers consisting of restriction fragment length polymorphisms (RFLPs), simple sequence repeats, and bin mapped expressed sequence tags (ESTs). Colinearity between wheat 1BS and rice was determined by aligning EST and RFLP probe sequences to the rice genome. Overall, colinearity was poorly conserved due to numerous complex chromosomal rearrangements, and of 48 wheat EST-RFLP sequences mapped, 30 had significant similarity to sequences on nine different rice chromosomes. However, 12 of the wheat sequences had similarity to sequences on rice chromosome 5 and were in a colinear arrangement with only a few exceptions, including an inversion of the markers flanking *Snn1*. High-resolution mapping of the *Snn1* locus in 8510 gametes delineated the gene to a 0.46-cM interval. Two EST-derived markers that cosegregated with *Snn1* were found to share homology to nucleotide binding site–leucine rich repeat–like genes and are considered potential candidates for *Snn1*.

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Wheat (*Triticum* sp.) is attacked by a large variety of pathogens, the majority being of fungal origin. The fungus *Stagonospora nodorum* (Berk.) Castellani & E.G. Germano (telomorph *Phaeosphaeria nodorum*) causes Stagonospora nodorum blotch (SNB) on both common wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD genomes) and durum wheat (*T. turgidum* L., $2n = 4x = 28$, AABB genomes). Because it has the ability to cause yield losses up to 50% and negatively impact grain quality, SNB is considered a disease of major importance in wheat-growing areas (King et al., 1983; Scharen et al., 1985; Weber, 1922). The pathogen produces typical lens-shaped necrotic and chlorotic lesions on susceptible genotypes but small-restricted lesions on resistant genotypes.

To date, four host selective toxins (HSTs) produced by *S. nodorum* have been identified (Liu et al., 2004a, 2006; Friesen et al., 2006, 2007, 2008). The toxins, designated SnToxA, SnTox1, SnTox2, and SnTox3, were all shown to be proteinaceous in nature and interact with specific host sensitivity genes to cause necrosis. It has been hypothesized that the SnToxA gene was horizontally transferred from *S. nodorum* to the tan spot fungus (*Pyrenophora tritici-*

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Abbreviations: BAC, bacterial artificial chromosome; CS, Chinese Spring; CS-DIC 1B, Chinese Spring-*Triticum dicoccoides* 1B disomic substitution line; EST, expressed sequence tag; HST, host selective toxin; ITMI, International Triticeae Mapping Initiative; LOD, logarithm of odds; N1B T1D, nullisomic 1B tetrasomic 1D; NBS-LRR, nucleotide binding site–leucine rich repeat; NCBI, National Center for Biotechnology Information; NSF, National Science Foundation; PAC, P1-derived artificial chromosome; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNB, Stagonospora nodorum blotch; SSCP, single-stranded conformational polymorphism; SSR, simple sequence repeat; TC, tentative consensus.

repentis) circa 1940, which was about the time tan spot emerged as an important wheat pathogen (Friesen et al., 2006). The wheat *Tsn1* gene on the long arm of chromosome 5B confers sensitivity to both SnToxA and the *P. tritici-repentis*–produced ToxA (Ptr ToxA) (Liu et al., 2006). *Snn2* and *Snn3* confer sensitivity to SnTox2 and SnTox3, respectively, and lie on wheat chromosome arms 2DS (Friesen et al., 2007) and 5BS (Friesen et al., 2008).

SnTox1 was the first HST identified from *S. nodorum*. The toxin was partially purified and the host gene conferring sensitivity to the toxin was identified and designated as *Snn1* (Liu et al., 2004a). The *Snn1* locus was genetically mapped to the short arm of chromosome 1B using the International Triticeae Mapping Initiative (ITMI) population (Liu et al., 2004a). In the same study, Liu et al. (2004a) used a series of 1BS deletion lines to determine that *Snn1* was physically located in the major gene rich region distal to the 1BS.sat.18 deletion breakpoint. It was also shown that the wheat cultivar Chinese Spring (CS) was sensitive to SnTox1 but that CS nullisomic 1B tetrasomic 1D (N1B T1D) was insensitive. In addition, the substitution of the native pair of CS 1B chromosomes with a pair of 1B chromosomes from an insensitive accession of *T. dicoccoides* resulted in an SnTox1 insensitive genotype. These results indicated that insensitivity to SnTox1 was not governed by a gene product per se but rather the lack of a gene for sensitivity, which is a result very similar to that shown for the *Tsn1*–Ptr ToxA interaction by Anderson et al. (1999). Furthermore, Liu et al. (2004b) inoculated the ITMI population with conidia from the SnTox1-producing isolate and used quantitative trait locus analysis to show that a compatible *Snn1*-SnTox1 interaction explained as much as 58% of the variation in SNB susceptibility, indicating that this interaction played an important role in disease.

Because a well-established transformation protocol and a better transposon gene tagging system are lacking, gene isolation by map based cloning remains the best strategy for cloning genes in wheat. However, the large genome size of approximately 17,300 Mb (Bennett and Leitch, 1995) and 21 pairs of chromosomes representing three ancestral (A, B, and D) genomes with (80%) repetitive DNA (Wicker et al., 2001; SanMiguel et al., 2002) means that chromosome walking in wheat is a challenge. Nevertheless, numerous genes have been isolated using map-based methods in recent years (see Keller et al. (2005) for review), indicating the feasibility of cloning genes in wheat.

To achieve map-based cloning, the construction of a saturated linkage map of the target locus followed by the development of a high-resolution map with DNA markers is required.

As of January 2008, the wheat National Science Foundation (NSF)–funded expressed sequence tag (EST) project and other private entities have generated 1,051,196 ESTs from hexaploid wheat and other closely related species (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html). Qi et al. (2004) physically mapped more than 16,000 EST loci to specific chromosomal bins using the wheat chromosome deletion lines (Endo and Gill, 1996). These bin-mapped ESTs provide a rich source of markers for use in saturation and high-resolution mapping, comparative mapping and sequence analysis, and the identification of candidate genes.

A great amount of research has focused on the identification of plant genes involved in mediating resistance to a diverse array of pathogens, but little is known about plant genes required for susceptibility. Better understanding of the molecular interactions between the factors encoding host susceptibility and pathogen virulence requires isolation and characterization of genes encoding these factors from both host and pathogen. Toward this goal as it pertains to the wheat–*S. nodorum* pathosystem, our long-term objective is to isolate and characterize the wheat *Snn1* gene. Here, we describe the development of saturated and high-resolution maps of the *Snn1* locus and the identification of candidate genes. We also evaluated colinearity between the wheat region harboring *Snn1* and rice (*Oryza sativa* L.) to determine the feasibility of using rice for further genomic analysis and marker development, and we describe the identification and development of markers that may be suitable for marker-assisted selection of the recessive *snn1* allele, which confers insensitivity to SnTox1.

MATERIALS AND METHODS

Plant Material and SnTox1 Screening

A segregating F_2 mapping population was developed from the cross between the SnTox1-sensitive hexaploid wheat cultivar Chinese Spring and the insensitive CS–*T. dicoccoides* chromosome 1B disomic substitution line (CS-DIC 1B). The CS-DIC 1B substitution line was maintained and provided by the Wheat Genetics and Genomics Resource Center at Kansas State University (Manhattan, KS). Saturation mapping was performed using 120 F_2 plants, and more than 16,000 F_2 plants were screened for reaction to SnTox1 for high-resolution mapping. Plants were grown in the greenhouse at an average temperature of 21°C with a 12-h photoperiod. The fully expanded secondary leaf was infiltrated with SnTox1 cultures that were partially purified as described by Liu et al. (2004a). Leaves were evaluated 3 d after infiltration and scored as either insensitive or sensitive.

In addition to CS and CS-DIC 1B, eight wheat lines were used to further evaluate the simple sequence repeat (SSR) markers that flank *Snn1*, including the genetic stock CS N1B T1D, the common wheat lines 'BR34' and ND495, the durum wheat lines 'Altar84', 'Divide', and 'Langdon', and the synthetic hexaploid wheat lines TA4152-60 and W-7976. CS, Altar84, Divide, TA4152-60 and W-7976 are sensitive to SnTox1 whereas CS-DIC 1B, N1B T1D, BR34, ND495, and Langdon are insensitive.

Saturation Mapping of the *Snn1* Locus

DNA from the parents and the population of 120 F_2 plants was isolated according to Faris et al. (2000).

The sequences of 120 ESTs that mapped to the 1BS. sat.18-0.50-1.00 deletion bin were downloaded from http://wheat.pw.usda.gov/cgi-bin/westsql/est_fasta.cgi. Primers for each EST were designed using Primer3 (Rozen and Skaletsky, 2000) and were amplified on parental DNA using the polymerase chain reaction (PCR) conditions outlined in Lu et al. (2006). Amplified products were separated on 6% MDE (Cambrex BioScience Rockland, Inc., Rockland, ME) gels to check for single stranded conformational polymorphisms (SSCPs) as described by Lu et al. (2006). For those EST primer pairs that showed no polymorphism between the parents, the amplified products were digested with the restriction enzyme *Rsa*I and separated on 6% MDE gels. If EST primer pairs yielded no amplification or if no polymorphism was observed using the methods described above, their PCR products were used as probes and mapped as restriction fragment length polymorphism (RFLP) markers as described by Lu et al. (2006). For RFLP analysis, 25 µg of parental DNA was digested with 10 different restriction enzymes (*Apa*I, *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Sac*I, *Sca*I, and *Xba*I). Restriction patterns that revealed parental polymorphisms were selected, and the corresponding restriction enzyme was used to map the polymorphic markers in the F_2 population. Restriction digestion and Southern hybridization were performed according to Faris et al. (2000).

In addition to the EST-derived markers from 1BS. sat.18-0.50-1.00, seven RFLP probes (KSUD14, KSUE19, KSU941, BCD22, BCD1124, CDO99, and CDO1340), and two ESTs, BE403631 (mapped to the deletion bin 1DS and 1AS) and BE637553 (mapped to the deletion bin 1DS), were mapped in the population using the methods described above as SSCP and RFLP markers, respectively. Four SSR markers (*Xfcp618*, *Xpsp3000*, *Xgwm11*, and *Xgwm273*) were also mapped in the population using the PCR conditions described by Röder et al. (1998). Amplified products from SSR primers were electrophoresed through 6% nondenaturing polyacrylamide gels, stained with SYBR Green II (Sigma, St. Louis) and visualized using a Typhoon 9410 variable mode imager (GE Healthcare, Waukesha, WI).

High-Resolution Mapping of the *Snn1* Locus

Of the more than 16,000 F_2 plants derived from CS × CS-DIC 1B that were screened for reaction to SnTox1, a total of 4255 were insensitive to the toxin. These 4255 insensitive plants were used as the high-resolution mapping population because they are homozygous for the recessive *snn1* allele and progeny testing was not necessary to determine their genotype. DNA was isolated from these plants following the high-throughput DNA extraction protocol developed by Bodo Slotta et al. (2008), with minor modifications adapted to using a Matrix Platemate Plus robot (Matrix Technologies Corporation, Hudson, NH). Fluorescent-labeled primer pairs for the SSR markers *Xpsp3000* (Bryan et al., 1997; http://wheat.pw.usda.gov/cgi-bin/graingenes/report.cgi?class=marker&name=PSP3000) and *Xfcp618* (L. Reddy and J.D. Faris, unpublished data), which flank *Snn1* based on the low-resolution map, were obtained. The FCP618 primer sequences are 5′-TCTA-CATACGGACTGAAATGGATAC-3′, and 5′-CCTGAT-TGAGACTCTGGTTACATAAGACTACTC-3′. The amplification was performed in 10-µL reaction consisting of 100 ng of template DNA, 1.5 mM $MgCl_2$, 0.125 mM dNTPs, 3 pmol of each forward and reverse primer, and 1 unit of DNA polymerase (Bioline Inc., London). Thermal cycling conditions were 94°C for 4 min followed by 34 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 2 min, followed by one cycle of 72°C for 7 min. Amplified products were then multiplexed and separated using an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA), and the fragment analysis and allele calling were performed according to Chao et al. (2007). Both the PCR set-up and gel electrophoresis were performed in 384-well plate format, with liquid handling done using the robot. Plants with recombination events between *Snn1* and either *Xpsp3000* or *Xfcp618* were used for high-resolution mapping of the markers within the interval defined by *Xpsp3000* and *Xfcp618* based on low-resolution mapping.

Linkage Analysis

The computer program MAPMAKER (Lander et al., 1987) V2.0 for Macintosh was used to calculate linkage and determine the order of markers on 1BS using a logarithm of odds (LOD) threshold of 3.0. The most likely order was determined using the "First Order" command and verified using the "Ripple" command. CentiMorgan distances were calculated using the Kosambi mapping function (Kosambi, 1944). Markers that did not "Ripple" at LOD > 3.0 were placed in their most likely positions on the map.

BLAST Similarity Searches

The sequences of RFLP and EST markers that were mapped in the CS × CS-DIC 1B mapping population were subjected to searches of The Dana-Farber Cancer Institute wheat gene index database (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/Blast/index.cgi) using BLASTn (Altschul et al., 1997) to identify corresponding tentative consensus (TC) sequences. The TC sequences (or EST sequences when no TC was found) were used as queries in tBLASTx searches of the National Center for Biotechnology Information (NCBI) nonredundant database (http://www.ncbi.nlm.nih.gov/) to identify the putative corresponding protein based on the criteria as described by Lu and Faris (2006). For comparison with rice, TC or EST sequences were subjected to BLASTn and tBLASTx searches of the rice genomic sequences using Gramene (Ware et al., 2002; http://www.gramene.org/Multi/blastview), genomic sequences of genes in rice pseudomolecules (TIGR Rice Genome Annotation Project, http://tigrblast.tigr.org/euk-blast/index.cgi?project=osa1), and all rice bacterial artificial chromosome (BAC)–P1-derived artificial chromosome (PAC) sequences in GenBank (http://tigrblast.tigr.org/euk-blast/index.cgi?project=osa1) using the criteria described by Lu and Faris (2006).

RESULTS

Saturation Mapping and Marker Analysis

Primers were developed for 117 of the 120 ESTs in the 1BS.sat.18 deletion bin. Only 9 (7%) of the 117 ESTs were mapped as SSCP markers with no restriction digestion of the PCR products with *Rsa*I, but the PCR product of one EST (BE406612) exhibited polymorphism when digested with *Rsa*I. The remaining ESTs were surveyed for RFLPs between the parents. Results indicate that 31 of them exhibited polymorphism. Thus, a total of 41 (35%) of the ESTs were mapped in the CS × CS-DIC 1B population either as SSCP or as RFLP markers (Table 1).

Three EST markers (*XBE498831*, *XBF474204*, and *XBF293222*) cosegregated with *Snn1* (Fig. 1). Three other EST markers (*XBE422980*, *XBE637568*, and *XBE605202*) cosegregated with each other 0.4 cM proximal to *Snn1*, and two EST markers (*XBF145399* and *XBF482862*) cosegregated with each other on the distal side of *Snn1*.

One EST marker, *XBE591617*, detected two loci (*XBE591617.1* and *XBE591617.2*) (Fig. 1), and both mapped to the *Snn1* proximal end. The ESTs BE403631 and BE637553 were reported by the wheat NSF-EST project to detect loci on the short arms of chromosomes 1A and/or 1D, but not 1B. Our results indicate that both ESTs detected fragments on 1BS. Also, although EST

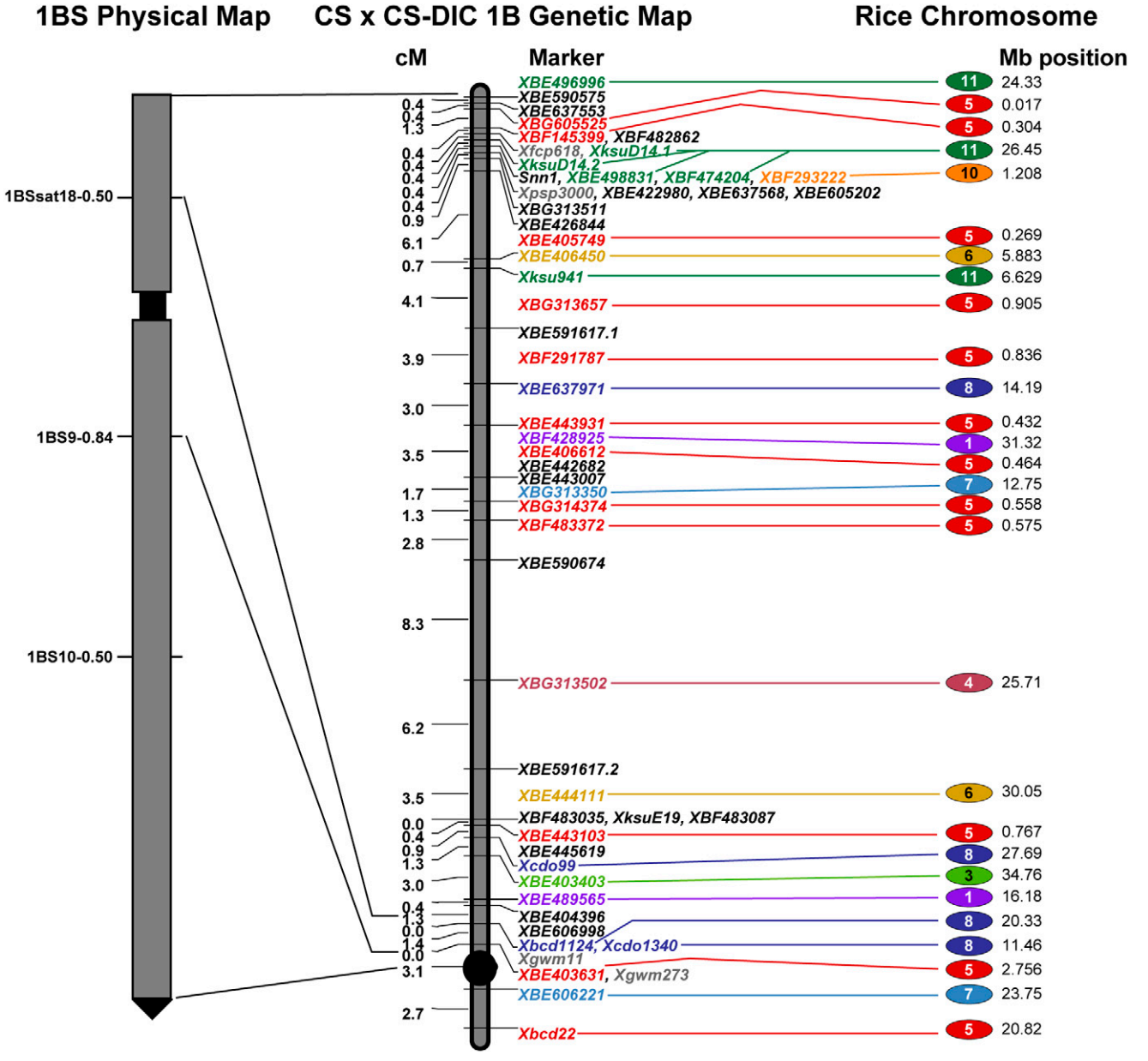

Figure 1. Low-resolution genetic linkage map of wheat chromosome arm 1BS (middle), the corresponding physical map based on deletion lines (left), and comparison of the marker sequences with rice (right). Along the genetic map, centiMorgan distances are shown to the left and markers to the right. Restriction fragment length polymorphism (RFLP) and expressed sequence tag (EST)-derived markers in different colors correspond to sequences on different rice chromosomes. Simple sequence repeat markers are shown in gray. Sequences of RFLP and EST-derived markers shown in black have no similarity to rice sequences.

Table 1. Expressed sequence tags (ESTs) mapped in the 'Chinese Spring' × Chinese Spring–*Triticum dicoccoides* 1B disomic substitution line population, the primer pairs used to evaluate them as single-stranded conformational polymorphism (SSCP) markers, and the marker systems used to map them.

GenBank accession	Polymerase chain reaction primers	Annealing temp.	Marker type for mapping (enzyme)
		°C	
BE406612	GCCCAAACTGAGACTACAAATACAT TCTTCCTCCAGTCCACAGACTTGG	55	SSCP (*Rsa*I)
BE637568	GCAAAAACAATTGGTACAAGGTACT TGCATAATATTGTGGTTAGGAATGA	53	RFLP† (*Bgl*II)
BE422980	CCATCTCTACAACAACAGGTGAAC AACTAAGCAACGATGATCCATCTAC	55	SSCP
BE403403	AAAGTACATCTCCTCTCTGGGTAAA CATCAACTACTACCGCAACCTG	55	SSCP
BE404396	GTATCTTGTGGGTGACTGATCAAC GCAGCAAAGTATTAACTCCGAT	55	RFLP (*Bam*HI)
BF293222	GGTTTGCTTTTGCCAATTGTTCTTG TATATGTTGGATGGGAGCAAAATCC	55	RFLP (*Bam*HI)
BE406450	CATTGAGCAGTTTCTCAAGGATAGT ATACTTGTTTAGCTCTGCGCCTAC	55	RFLP (*Hin*dIII)
BE442682	GAGCCTGATTGCCTATAAATACAGA ACGCACTCTGACTTATTCCATTATC	55	RFLP (*Eco*RV)
BE590674‡	AGCAACAACAACAACCAATTCTACT GAGGTTGTTGGAAAGAACACTGTC	55	RFLP (*Eco*RI)
BG313350	GATGATTATCGACCTCCAGGAATAA GTAACAGAAAGTTTTCCCTCAGGTT	55	RFLP (*Hin*dIII)
BF145399	GCCCTACTTCCTCTTCGACGGCTAC AAATACAACAGGTGCTAGGTACGAC	58	RFLP (*Eco*RI)
BE489565	GCTAACATAAAGACATGGACCTTCAC TGTATCAGGAAGTATCCTCCACTCT	55	RFLP (*Bam*HI)
BE443007	GTGTGTGAGTAGTACGTGTGTGTGC GCGTGATGAATACTTAGATGACTTAC	55	RFLP (*Eco*RV)
BE444111	ACCTATTTCCTCCGCTCCACCAAC CGAATATATTACTCCCTCCGTATCA	53	RFLP (*Bam*HI)
BE443931	ACTATCATGCAATCTGGTTCAGTAG ACCTATTTCCTCCGCTCCACCAAC	55	RFLP (*Bam*HI)
BE606221	CCTCTCGACGGCAGAACCTAGCC GTGTTCTCTCTGAAGAATTCTTGG	53	SSCP
BE606998	GCACACCAACTGGATAATCCC GGATATCCCCATTAAAAGGAAG	50	RFLP (*Eco*RV)
BE605202	AATCTGAAATCTATACATGCCTTGG CAGCTTTGATCACAAGTGGAAGT	53	RFLP (*Bgl*II)
BE443103	AACAAAAAGGCTTCTGAAGTCATAG GAGCTTGTGTCGTCACTAGGTTTAT	55	RFLP (*Hin*dIII)
BE637971	ATCACCAGCTGTTAAAGAGTGATTC CAAATATATGCCTCTTCTTGTCCAG	55	RFLP (*Hin*dIII)
BG313511	CGAGGACGAACTAATTAAGCAAGGA TCAACAACTGACTACTTGAAGAACG	55	RFLP (*Eco*RI)
BG313657	GGCTGTCCTTGATATGCAACTCTAT CTGGAGAAAAAGTAGAGCAACGACT	58	RFLP (*Bam*HI)
BG314374	ACTAGAAGCTACAACCGGGATAGAT CTACAGTGCCTCTCAGTAGACTCG	58	SSCP
BE445619	GTGTGTGTCAGCTAATGGTATTACG GTGCTCTGACTTGTGTCACTGTATC	55	RFLP (*Hin*dIII)
BE591617	GCAGAACGGAAATAGCTTTTGTGAA TATACGCTTAAGAGACATGGAGCAG	55	RFLP (*Hin*dIII)
BF482862	TTTTTGGAGGAACAGAACAATATAA GATAGTGAGTCCTGCACGGTTAC	52	RFLP (*Bam*HI)
BF483035	CTCTCCTCCGATCTCTCGTGTTAT TCTGCACATGACTGCCTATAGATAC	55	RFLP (*Hin*dIII)
BF483087	CAGAGGATTACAGCCTAAAG GAATGATGGTGTTTCAGATG	50	SSCP
BE590575	CAACAACAATTACCACAACAACAAT TACCAACAATATCCACAACAACAAC	55	RFLP (*Eco*RI)
BF291787‡	CAATTCAATGAAGGCATTTCCTCC ACTGGGAACTTTGTATTATGACAGC	55	RFLP (*Bam*HI)
BG313502	CTGAAGCTTATGTCCTCAACATTC CAAGTACTCAGTTCCAAGCTCCTT	55	RFLP (*Eco*RI)
BE496996	CTTTGGTTCAATGTCCAATGTGCC ATCACTTTCTCCATTGCTATCTCC	55	SSCP
BE405749	ACCATGGAGAATGACTTTGTAG GGATCAGCTATAGGATTATCAG	52	RFLP (*Eco*RI)
BE426844	CTATGAGCTCCACAGATGTCCCAGC CGCATGCCATTCTCTAATTTTGTGG	58	RFLP (*Eco*RV)
BE498831	ATTTTCAGGAGTTAGTGTCATGCTC GTTAGTGTGCTTGGTAAAATTACGG	55	RFLP (*Bam*HI)
BF474204	AATCACACGACCCAGTAAGTTCTC CTCAAGTACCTCTGCTTCAACTTC	55	SSCP
BF428925	CAGTACTGCTCTGCTTGAGATG CTGTAACAAACATCAGAGGGCA	55	RFLP (*Bam*HI)
BG605525	CAGAGCGTTTGCATAATACAG CTCGAATTTGGTGTTGTCAGA	52	RFLP (*Dra*I)
BF483372	CGGGTGAAATGCTACATTGTTAATG GGTTGCATTTGAAGCACAGACCCG	55	SSCP
BE40363§	TTCTCTATGTCGGGATGGATGC AAGGTTTGTTTGGGGGGTGAGC	55	SSCP
BE637553¶			RFLP (*Dra*I)

†RFLP, restriction fragment length polymorphism.

‡No amplification product was observed for these ESTs, but they were mapped as RFLP markers.

§ National Science Foundation EST deletion mapping project mapped this EST to the deletion bin 1DS and 1AS but not to 1BS.

¶ National Science Foundation EST deletion mapping project mapped this EST to the deletion bin 1DS but not to 1BS.

BE606221 was reported to be in the 1BS.sat.18 deletion bin, it mapped near the centromere in our population. It is possible that fragments different from those that were physically mapped by the wheat NSF-EST project were polymorphic in our population.

Of the seven RFLP probes tested, KSUD14 detected fragments most closely linked to *Snn1*. KSUD14 contains a fragment of the *Lr21* gene from chromosome arm 1DS (Huang et al., 2003), which confers resistance to the wheat leaf rust pathogen (*Puccinia triticina* Eriks). It detected multiple loci, two of which were polymorphic

and mapped distal to *Snn1* as markers *Xksud14.2* and *Xksud14.1* at genetic distances of 0.4 and 0.8 cM, respectively (Fig. 1). Of the four SSR markers, *Xfcp618* and *Xpsp3000* were the most closely linked to *Snn1*. *Xfcp618* cosegregated with *XksuD14.1* 0.8 cM on the distal side of *Snn1*, and *Xpsp3000* mapped 0.4 cM proximal to *Snn1*.

The genetic linkage map of chromosome 1B generated in the CS × CS-DIC 1B population of 120 F_2s contained a total of 54 markers spanning 64.6 cM (Fig. 1). Forty-two of the markers mapped at LOD ≥ 3.0; the remaining 12 were placed at the most likely positions along the map. The segment of the linkage map corresponding to deletion bin 1BS.sat.18-0.50-1.00 contained 47 markers and spanned a genetic distance of 56.1 cM, representing an average density of 1 marker per 1.1 cM. Within a 1.6-cM region encompassing the *Snn1* locus, there were 11 markers, for an average of 1 marker per 0.15 cM.

Searches of the NCBI nonredundant database using the EST–TC and RFLP probe sequences as queries revealed that 29 sequences had significant homology to predicted proteins (Table 2). Of the three ESTs that cosegregated with *Snn1,* BE498831 and BF474204 had similarity to nucleotide binding site–leucine rich repeat (NBS-LRR)–like genes from *Aegilops tauschii* and BF293222 had similarity to a protein kinase from rice. The RFLP probes KSUD14 and KSU941 also had similarity to NBS-LRR–like genes from wheat.

Colinearity between 1BS and Rice

To evaluate colinearity between chromosome arm 1BS and rice, the sequences of all mapped RFLP and EST-based markers were subjected to BLAST searches against rice genomic sequences. The sequence for RFLP probe KSUE19 was not available, and KSUD14 and BE591617 each detected two loci; therefore, 47 sequences were evaluated. Of these, 30 had homology to rice genomic sequences on nine different chromosomes (Fig. 1). Among these, 12 marker sequences had similarity to sequences on rice chromosome 5. Comparison of the positions of the markers with similarity to sequences on rice chromosome 5 indicated a good level of colinearity with only a few exceptions (Fig. 2). Eight of the 12 markers were perfectly colinear, but markers *XBE313657* and *XBF291787* were inverted relative to each other and to the proximal five colinear markers *XBE443931*, *XBE406612*, *XBG314374*, *XBF483372*, and *XBE443103*. Also, markers *XBF145399* and *XBE405749*, which flank *Snn1*, were inverted relative to each other. The sequences of rice chromosome 5 detected by the 10 most distal markers were all within the first 1.0 Mb of the short arm of rice chromosome 5 corresponding to the wheat deletion bin 1BS.sat.18-0.50-1.00.

Five marker sequences had similarity to sequences on rice chromosome 11, but among these, *Xksud14*, *XBE498831*, and *XBF474204*, which all have high degrees of similarity to known NBS-LRR–like genes, all had similarity to the same NBS-LRR–like gene on rice chromosome 11. Therefore, only three unique sequences of rice chromosome 11 were detected by the five marker sequences.

Four marker sequences had similarity to rice chromosome 8 sequences, and the remaining nine detected sequences on rice chromosomes 1 (2), 3 (1), 4 (1), 6 (2), 7 (2), and 10 (1) (Table 2, Fig. 1). Seventeen markers had no significant similarity to any rice sequences. Of these, only five had significant similarities to known proteins (Table 2).

High-Resolution Mapping

The 4255 insensitive F_2 plants were screened with the SSR markers *Xfcp618* and *Xpsp3000*, which closely flank *Snn1*, to identify recombinants for high-resolution mapping. A population of this size allows the analysis of 8510 gametes and provides a resolution of 0.01 cM. *Xfcp618* behaved as a dominant marker and detected a 250-bp fragment in CS, but no amplification was observed in CS-DIC 1B (Fig. 3). *Xpsp3000* behaved as a codominant marker and detected a 281-bp fragment in CS and a 252-bp fragment in CS-DIC 1B. Screening of the 4255 plants resulted in the identification of 63 recombinants between *Xfcp618* and *Snn1* and 14 recombinants between *Xpsp3000* and *Snn1*. Therefore, *Xfcp618* and *Xpsp3000* flanked *Snn1* at genetic distances of 0.74 and 0.16 cM in the high-resolution population (Fig. 4).

We mapped *Xksud14.1*, *XksuD14.2*, *XBE498831*, *XBF474204*, *XBF293222*, *XBE422980*, *XBE637568*, and *XBE605202* in the population of 77 recombinants. The results indicate that *Xksud14.1* cosegregated with *Xfcp618* at 0.74 cM from *Snn1*, whereas *Xksud14.2* mapped 0.34 cM distal to *Snn1* (Fig. 4). Marker *XBF293222*, which cosegregated with *Snn1* in the low-resolution population, mapped 0.12 cM proximal to *Snn1*. Markers *XBE422980*, *XBE637568*, and *XBE605202*, which cosegregated with *Xpsp3000* in the low-resolution population, also cosegregrated with *Xpsp3000* in the high-resolution population. It is possible that these three markers actually map proximal to *Xpsp3000*, but because we used *Xpsp3000* to select for recombinants, our recombinant population of 77 plants did not take into account recombination events proximal to *Xpsp3000*.

These results delimit *Snn1* to a 0.46-cM interval flanked by *Xksud14.2* and *XBF293222* (Fig. 4). Markers *XBE498831* and *XBF474204* cosegregated with *Snn1*, indicating that these two ESTs, which have similarity to NBS-LRR–like resistance genes could be considered as putative candidates for *Snn1*.

Evaluation of SSR Markers Flanking *Snn1* in Ten Wheat Lines

In addition to CS and CS-DIC 1B, the flanking SSR markers *Xfcp618* and *Xpsp3000* were evaluated in eight additional wheat lines, including N1B T1D, used as a negative control (Fig. 3). The FCP618 primer set detected a 250-bp fragment in all five *Snn1*-containing lines, and three different alleles were observed among the SnTox1 insensitive lines, including a null allele in CS-DIC 1B. BR34 and ND495 each had a 265-bp allele, and Langdon

Table 2. Expressed sequence tag (EST) markers mapped to the *Snn1* region with their corresponding tentative consensuses (TCs), putative function based on tBLASTx searches against the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/) and homology to rice genomic sequences and their possible chromosomal (chr.) locations based on BLASTn and tBLASTx searches against Gramene rice genomic sequences, The Institute for Genomic Research (TIGR) genes in rice pseudomolecules and rice bacterial artificial chromosome–P1-derived artificial chromosome sequences.

GenBank accession	Marker	TC	NCBI tBLASTx hit	e-value	Rice BLASTn		Rice tBLASTx	
					Rice chr.	e-value	Rice chr.	e-value
BE496996	*XBE496996*	N/A[†]	NS[‡]		11	e-23	11	e-32
BE590575	*XBE590575*	TC250042	NS		NS		NS	
BE637553	*XBE637553*	N/A	NS		NS		NS	
BG605525	*XBG605525*	TC252893	Hypothetical protein (*Oryza sativa*)	e-122	5	e-143	5	e-73
BF145399	*XBF145399*	TC249015	Putative vacuolar ATP synthase (*Oryza sativa*)	e-59	5	e-158	5	e-48
BF482862	*XBF482862*	TC244170	NS		NS		NS	
AB325481[§]	*Xksud14*	N/A	*Lr21* gene (*Triticum aestivum*)	e-119	11	e-28	11	e-177
BE498831	*XBE498831*	N/A	NBS-LRR (*Aegilops tauschii*)	0	NS		11	e-27
BF474204	*XBF474204*	TC260263	NBS-LRR class RGA/*Lr21* (*Aegilops tauchii*)	e-102	11	e-15	11	e-74
BF293222	*XBF293222*	N/A	Putative protein kinase (*Oryza sativa*)	e-52	10	e-78	10	e-76
BE422980	*XBE422980*	TC233421	Gamma-gliadin (*Triticum aestivum*)	e-49	NS		NS	
BE637568	*XBE637568*	TC264054	Avenin (*Avena sativa*)	e-24	NS		NS	
BE605202	*XBE605202*	TC238750	NS		NS		NS	
BG313511	*XBG313511*	TC260102	NS		NS		NS	
BE426844	*XBE426844*	TC266174	Putative proteinase inhibitor (*Hordeum vulgare*)	e-21	NS		NS	
BE405749	*XBE405749*	TC270195	Hypothetical protein (*Oryza sativa*)	0	5	e-260	5	e-110
BE406450	*XBE406450*	TC252657	12-oxo-phytodienoic acid reductase (*Zea mays*)	0	6	e-58	6	e-138
AF445764[§]	*Xksu941*	N/A	NBS-LRR like protein (*Triticum aestivum*)	0			11	e-41
BG313657	*XBG313657*	TC247637	Unknown protein (*Oryza sativa*)	0	5	0	5	e-205
BE591617	*XBE591617*	TC273936	NS		NS		NS	
BF291787	*XBF291787*	TC238176	Putative MAP kinase phosphatase (*Oryza sativa*)	e-130	5	e-270	5	e-131
BE637971	*XBE637971*	TC269184	Nuclear inhibitor of PP1-like (*Oryza sativa*)	e-99	8	e-150	8	e-94
BE443931	*XBE443931*	TC255562	Hypothetical protein (*Oryza sativa*)	e-18	5	e-30	5	e-12
BF428925	*XBF428925*	TC273436	Putative elongation factor 2 (*Oryza sativa*)	e-71	1	e-31	1	e-63
BE406612	*XBE406612*	TC250410	Putative cysteine protease (*Oryza sativa*)	e-155	5	e-179	5	e-139
BE442682	*XBE442682*	TC249380	Chymotripsin inhibitor-2A (*Hordeum vulgare*)	e-34	NS		NS	
BE443007	*XBE443007*	TC256524	NS		NS		NS	
BG313350	*XBG313350*	TC263654	Putative CLB1 protein (*Oryza sativa*)	0	7	e-291	7	e-120
BG314374	*XBG314374*	N/A	NS		5	e-23	5	e-16
BF483372	*XBF483372*	TC241450	Hypothetical protein (*Oryza sativa*)	e-18	5	e-31	5	e-32
BE590674	*XBE590674*	TC250064	LMW glutenin subunit (*Triticum turgidum*)	e-31	NS		NS	
BG313502	*XBG313502*	N/A	Phenylalanine ammonia-lyase (*Triticum aestivum*)	0	4	e-83	4	e-59
BE444111	*XBE444111*	TC269874	Hypothetical protein (*Oryza sativa*)	e-20	6	e-19	6	e-19
BF483035	*XBF483035*	TC255783	NS		NS		NS	
BF483087	*XBF483087*	TC236406	NS		NS		NS	
BE443103	*XBE443103*	TC256237	Hypothetical protein (*Oryza sativa*)	e-63	5	e-44	5	e-48
BE445619	*XBE445619*	TC242559	NS		NS		NS	
BE439326[§]	*Xcdo99*	TC265425	NS		8	e-109	8	e-60
BE403403	*XBE403403*	TC257808	Putative hydrolase (*Oryza sativa*)	e-73	3	e-61	3	e-45
BE489565	*XBE489565*	TC238829	NS		1	e-50	1	e-72
BE404396	*XBE404396*	N/A	NS		NS		NS	
BE606998	*XBE606998*	TC259788	NS		NS		NS	
BE439012[§]	*Xbcd1124*	TC254106	Annexin (*Triticum aestivum*)	0	8	e-37	8	e-96
CN180769[§]	*Xcdo1340*	N/A	NS		8	e-98	8	e-51
BE403631	*XBE403631*	N/A	Na+/H+ antiporter (*Triticum aestivum*)	0	5	e-110	5	e-54
BE606221	*XBE606221*	TC248370	Calcium binding protein (*Oryza sativa*)	e-173	7	e-294	7	e-104
BE438918[§]	*Xbcd22*	N/A	NS		5	e-63	5	e-47

[†]N/A, not available.

[‡]NS, nonsignificant.

[§]Restriction fragment length polymorphism probes for which the sequence was obtained from http://www.ncbi.nlm.nih.gov/.

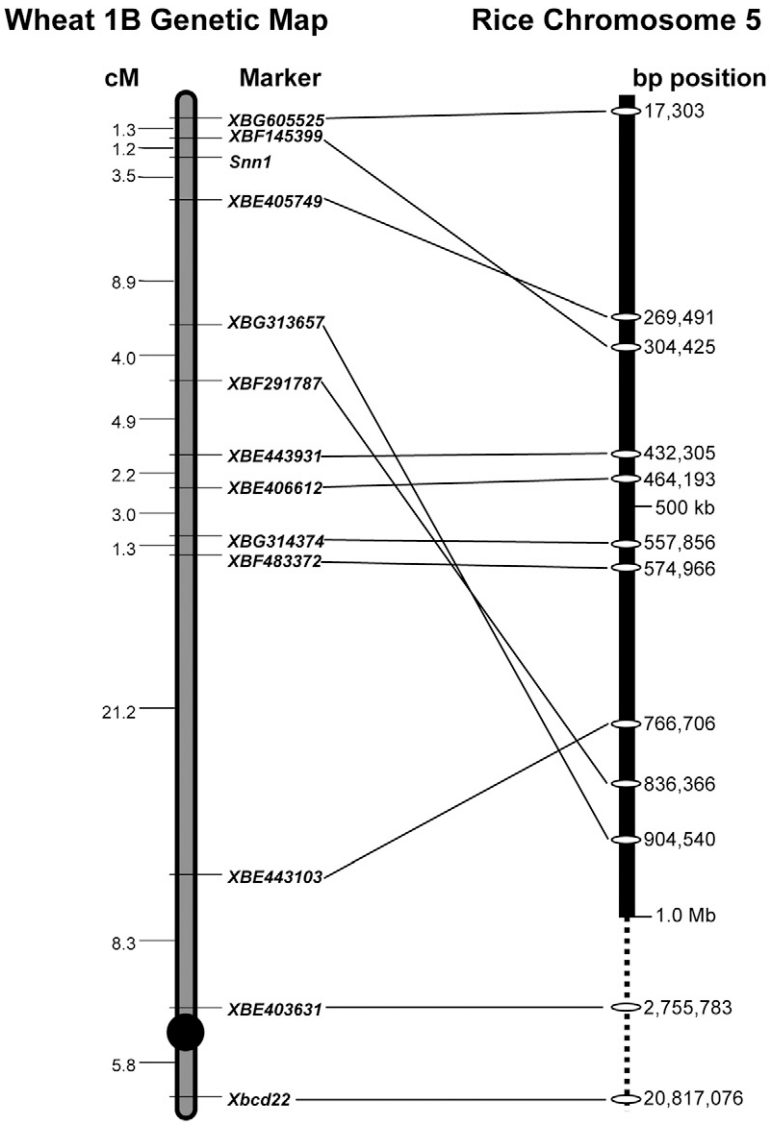

Figure 2. Colinearity between wheat chromosome arm 1BS and rice chromosome 5. Indicated to the left is the genetic map of 'Chinese Spring' × Chinese Spring–*Triticum dicoccoides* 1B disomic substitution line containing only markers that had significant homology to rice sequences on chromosome 5. To the right is rice chromosome 5 indicating the corresponding genomic location for wheat markers and the megabase positions. The thick black line represents 1 Mb and is drawn to scale. The dotted line represents beyond 1 Mb and is not to scale.

possessed two fragments, which were approximately 254 and 249 bp. The PSP3000 primer set detected a 250-bp fragment in all the SnTox1 sensitive lines except for CS, where it detected a 281-bp fragment. Among the SnTox1 insensitive lines, PSP3000 detected fragments of 254, 252, 215, and 210 bp in BR34, CS-DIC 1B, Langdon, and ND495, respectively.

DISCUSSION

The wheat pathogen *Stagonospora nodorum* causes SNB on wheat wherein multiple HSTs are involved in disease development (Friesen et al., 2007, 2008). These toxins are each recognized directly or indirectly by specific genes in the host, leading to compatible interactions and the development of disease. To date, four wheat–*S. nodorum* HST interactions have been characterized, and it has been shown that SnTox1 interacts with *Snn1* (Liu et al., 2004a,b), SnToxA interacts with *Tsn1* (Friesen et al., 2006; Liu et al., 2006), SnTox2 interacts with *Snn2* (Friesen et al., 2007), and SnTox3 interacts with *Snn3* (Friesen et al., 2008), indicating that multiple gene-for-gene interactions govern susceptibility of wheat to SNB. Understanding the molecular mechanism of host sensitivity to the toxins produced by *S. nodorum* requires the isolation of genes conditioning sensitivity in the host. Here, we developed saturated and high-resolution genetic maps of the *Snn1* locus, assessed colinearity with rice, and identified markers closely flanking *Snn1*, which are prerequisites for positional cloning.

The previous physical mapping of *Snn1* to deletion bin 1BS.sat.18 (Liu et al., 2004a) allowed us to exploit 117 ESTs that were bin-mapped by the wheat NSF-EST project. Not only did the use of bin-mapped ESTs provide a rich source of markers targeted to a chromosomal region of interest, but because they are derived from expressed genes, their sequence information can be exploited to obtain evidence of function, and they are ideal for conducting comparative mapping and colinearity studies. Of the 52 markers that mapped to the short arm of chromosome 1B in the CS × CS-DIC 1B population, 47 mapped within a 56.1-cM segment that corresponded to deletion bin 1BS.sat.18, which accounts for about 12% of the physical size of the arm. Five markers spanning only 2.7 cM mapped in the segment of the genetic map that corresponded to the rest of 1BS, which accounts for about 88% of the physical size of the arm. This agrees with previous studies in that a vast majority of recombination that occurs on wheat homeologous group 1 short arms occurs in the most distal portions (Gill et al., 1996b; Sandhu et al., 2001; Erayman et al., 2004). For example, Sandhu et al. (2001) reported that 85% of the recombination along group 1 short arms occurred in the "1S0.8" region, which corresponds to the 1BS.sat.18 deletion bin, and the remaining 15% occurred along the rest of the chromosome. Uneven distribution of recombination has been well documented in wheat and other organisms (Gill et al., 1993, 1996a,b; Tranquilli et al., 1999; Faris et al., 2000; Spielmeyer et al., 2000; Lu et al., 2006).

Studies have also reported that the genes in cereals are present in clusters encompassing physically small chromosomal regions (Gill et al., 1993, 1996a,b; Civardi et al., 1994; Descenzo et al., 1996; Wei et al., 1999) and that the distal regions of the short arms of wheat homeologous group 1 chromosomes are very gene rich (Gill et

al., 1996b; Sandhu et al., 2001; Brooks et al., 2002; Erayman et al., 2004; Peng et al., 2004). Sandhu et al. (2001) reported that 75% of markers used in their study derived from expressed sequences mapped to the 1S0.8 region. Analysis of the *Lrk* gene-containing region at the distal end of 1AS indicated that gene density was as high as one gene per 5 to 20 kb (Feuillet and Keller, 1999).

Therefore, our data indicate that *Snn1* lies within a gene-rich recombination hot spot of the wheat genome. The 1BS.sat.18 deletion bin where *Snn1* resides represents the distal half of the chromosome 1BS satellite, which is approximately 0.5 µm in length and contains 34 Mb of DNA (Endo and Gill, 1996; Gill et al., 1996b). Taking into account that we have mapped 47 markers to this bin gives an average density of one marker per 723 kb, and because these markers accounted for 56.1 cM of genetic distance, the average physical to genetic distance ratio across the bin is 607 kb per cM, which makes the recombination frequency in this region sevenfold higher than the whole genome average of 4.4 Mb cM^{-1} (Faris et al., 2000).

Colinearity between *Snn1* and rice was studied to determine if information from the rice genome could be exploited to conduct further genomic analysis of the *Snn1* region, develop additional markers, and possibly identify candidate genes. Our data indicate that of the 47 marker sequences evaluated, 30 (64%) had similarity to sequences on nine different rice chromosomes, but 12 (40%) of these had similarity to sequences on rice chromosome 5. Other studies have reported mosaic conservation of wheat group 1 chromosomes with rice but also that syntenic relationships exist between wheat homeologous group 1 chromosomes and rice chromosome 5 (Kurata et al., 1994; Van Deynze et al., 1995; Sorrells et al., 2003; Peng et al., 2004; Guyot et al., 2004). Sorrells et al. (2003) evaluated 12 ESTs mapped to 1BS.sat.18 and reported that 4 (33%) of them had similarity to sequences on rice chromosome 5, whereas the other 8 detected loci elsewhere in the rice genome. Peng et al. (2004) evaluated 2212 EST loci mapped using the homeologous group 1 deletion lines and reported that among 37 ESTs that mapped within the homeologous regions of the short arm terminal bins and had matches to rice sequences, 16 (43%) had similarity to sequences on rice chromosome five, whereas the other 21 had similarity to all the remaining 11 rice chromosomes with the exception of chromosome 9. Guyot et al. (2004) compared RFLP probe sequences from wheat 1AS with rice 5S and found good colinearity between the short arm of wheat chromosome 1A and rice chromosome 5. However, *in silico* comparative analysis of 1500 kb from physical contigs of wheat chromosome 1AS with rice chromosome 5S revealed much lower levels of colinearity. Therefore, our results regarding the colinearity between wheat chromosome arm 1BS and rice agree closely with others in that, overall, there is a low level of conservation between 1BS and rice, but some degree of colinearity between 1BS and rice chromosome 5 is evident.

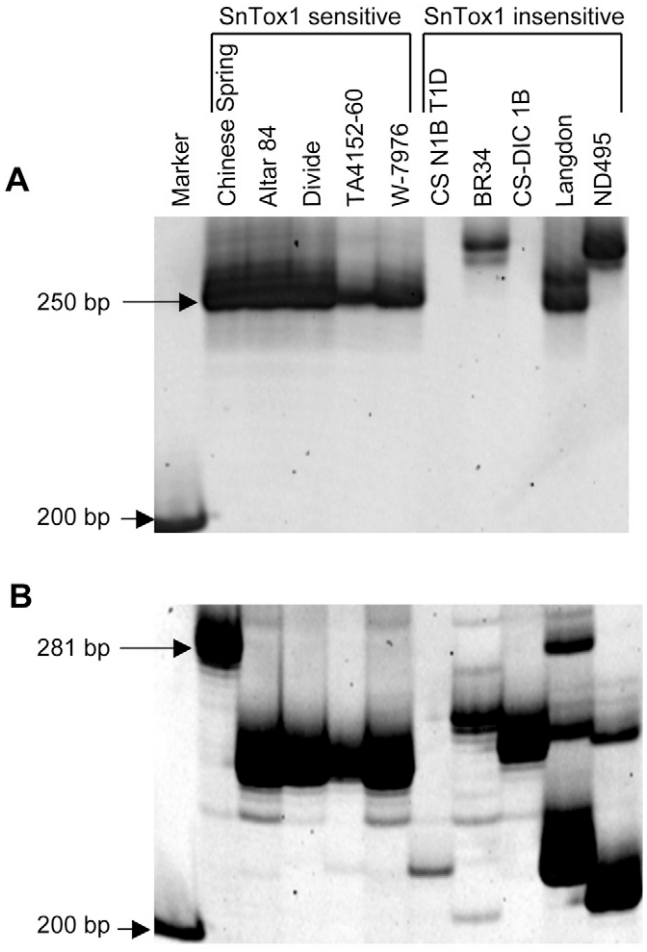

Figure 3. Polymerase chain reaction amplification of genomic DNA of 10 wheat lines with the simple sequence repeat primer sets (A) FCP618 and (B) PSP3000. Lanes are annotated across the top.

The disruptions in colinearity between 1BS and rice chromosome 5 are primarily due to inversions, duplications, gene movement, and other rearrangements. Numerous disruptions near the *Snn1* locus make it difficult to exploit the rice genome for further marker development and analysis of the *Snn1* locus. Within a 2.0-cM region encompassing the *Snn1* locus, there are 12 markers mapped, 2 of which are SSR markers (Fig. 1). Of the remaining 10, 4 (*XBF482862*, *XBE422980*, *XBE637568*, and *XBE605202*) had no similarity to rice sequences, 4 (*XksuD14.1*, *XksuD14.2*, *XBE498831*, and *XBF474204*) all had similarity to the same NBS-LRR–like gene on rice chromosome 11, 1 marker (*XBF145399*) had similarity to a sequence on rice chromosome 5, and 1 (*XBF293222*) had similarity to a sequence on rice chromosome 10, indicating that colinearity with rice is not well conserved near the *Snn1* locus.

Our high-resolution genetic map delineated the *Snn1* locus to a 0.46-cM interval between the markers *Xksud14.2* and *XBF293222* (Fig. 4). Given that the average recombination frequency of the 1BS.sat.18 bin is estimated to be 607 kb cM^{-1}, the distance between the closest flanking markers would correspond to approximately

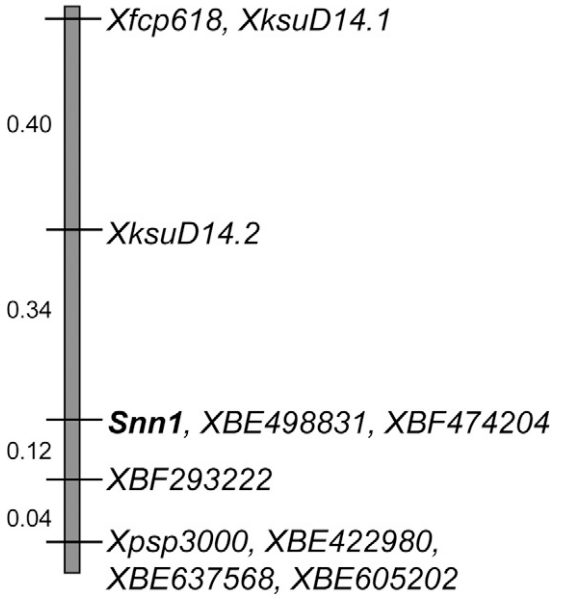

Figure 4. High-resolution genetic map of the *Snn1* locus developed using 4255 F_2 wheat plants (8510 gametes). Markers are shown along the right and genetic distances along the left of the map.

279 kb. Taking into account the two markers that cosegregated with *Snn1* gives an average marker density at the *Snn1* locus of one marker per 93 kb.

User-friendly PCR-based codominant markers are highly desirable for conducting high-throughput genotyping and marker-assisted selection. Basic attempts were made to convert the EST markers that were closely linked with, or cosegregated with, *Snn1* to more user-friendly platforms, but they were unsuccessful. However, *Xfcp618* and *Xpsp3000*, which delineate *Snn1* to a 0.9-cM segment, meet the criteria for high-throughput genotyping platforms and should be useful for such purposes. Marker-assisted selection of toxin insensitivity genes is especially efficient in backcrossing schemes because insensitivity is recessive and heterozygous genotypes cannot be distinguished from plants homozygous for the recurrent parent since both classes are sensitive. The use of marker-assisted selection allows one to distinguish these classes and select heterozygous genotypes for conducting further rounds of backcrossing.

The two ESTs BE498831 and BF474204, which cosegregated with *Snn1,* both had significant homology to NBS-LRR–like proteins (Table 2). NBS-LRR–like genes are viable candidates for genes governing sensitivity to the HSTs produced by *S. nodorum*. In oat (*Avena sativa* L.), the *Pc-2* gene confers resistance to the rust fungus *Puccinia coronata*, but *Pc-2*-containing oats are susceptible to Victoria blight caused by *Cochliobolus victoriae* and sensitive to the *C. victoriae*–produced toxin known as victorin (see Wolpert et al., 2002, for review). The rust resistance gene *Pc-2* and the victorin sensitivity gene *Vb* have not been separated genetically, indicating that either *Pc-2* has pleiotropic effects or there are two different but tightly linked genes. A gene designated *LOV1* was found to confer sensitivity to victorin in *Arabidopsis* and shown to be a CC-NBS-LRR–like gene (Lorang et al., 2007). Sequence analysis of loss-of-function mutants for *LOV1* indicated that the nucleotide binding domain shared by Apaf-1, certain R gene products, and CED-4 (NB-ARC) (P loop) domain and, to a lesser extent, the LRR domain were important for function (Sweat et al., 2008).

Other evidence of toxin sensitivity genes with disease resistance-like features has also been reported. Nagy et al. (2007) constructed a sorghum BAC contig spanning the *Pc* gene, which conditions sensitivity to the Pc-toxin, and identified an NBS-LRR gene as a likely candidate. Therefore, the evidence to date suggests that disease-resistance genes, particularly NBS-LRR–like genes, are probable candidates for toxin sensitivity genes, and the toxin-producing pathogens may have evolved mechanisms to exploit R-gene receptors to attack the cells and cause susceptibility. Given that two ESTs with homology to NBS-LRR–like genes cosegregate with *Snn1*, we consider them both viable candidates for *Snn1*, and they will be a focus of future research. However, it is important to note that, given our estimate of 279 kb for the physical distance between markers flanking the *Snn1* locus and the possibility that gene density could be as high as one gene per 5 to 20 kb in this region, it is possible that between 10 and 50 genes could be present within this interval. Although the NBS-LRR–like genes are attractive candidates, other candidate genes likely exist within the interval as well. Additional work, including the development of a BAC-based physical map and sequence analysis of the locus, is needed to determine the identity of the *Snn1* gene.

A well-understood system similar to SnTox1 is ToxA from *Pyrenophora tritici-repentis*. ToxA and SnTox1 are both proteins and light dependent, and both interact with dominant sensitivity genes in the host (Liu et al., 2004a; Faris et al., 1996; Anderson et al., 1999). Manning and Ciuffetti (2005) showed that ToxA is internalized, probably via receptor-mediated endocytosis, and localized to the chloroplasts. In the chloroplast, ToxA was found to interact with ToxABP1 protein required for PSII function and thylakoid integrity (Manning et al., 2007). It is probable that both SnTox1 and ToxA utilize the same, or similar, pathways once internalized within the plant cell. It is possible that NBS-LRR-like genes provide the specificity for recognition of the toxins. The isolation of toxin sensitivity genes such as *Snn1* and *Tsn1* in wheat will provide more insights regarding the mechanisms of toxin recognition and toxin-induced cell death, as well as how their function is analogous to that of resistance genes and the evolutionary events leading to toxin sensitivity.

CONCLUSIONS

Here, we described the genomic analysis of the *Snn1* locus on wheat chromosome arm 1BS. The use of unique wheat cytogenetic stocks such as CS N1B T1D and CS-DIC 1B allowed us to target markers to chromosome 1B and

develop and use low- and high-resolution populations segregating for only the target chromosome. The wheat bin-mapped ESTs provided a rich source of markers, and the finished rice genome sequence allowed us to conduct comparative studies using mapped wheat EST sequences. Because colinearity between rice and the wheat *Snn1* region appears to be poorly conserved, it will be difficult to use rice as a vehicle for cloning *Snn1*. However, through high-resolution mapping, we delineated *Snn1* to a 0.46-cM region and identified two ESTs, which we consider plausible candidates for *Snn1*. Therefore, the stage is set for the map-based cloning of *Snn1* using an appropriate BAC library, which will be a focus of future research.

Acknowledgments

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